

DOCTORAL THESIS

Roles of neurokinin receptor one in six-hydroxydopamine-lesioned rat: an animal model of Parkinson's disease

Chan, Wing Sai

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**Roles of Neurokinin Receptor One in Six-hydroxydopamine-lesioned
Rat: An Animal Model of Parkinson's Disease**

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ABSTRACT

Parkinson's disease is a serious motor disorder and it is caused by a degeneration of dopaminergic neurons in the substantia nigra pars compacta. Neurokinins (NKs) are a group of neuropeptides that are suggested to be involved in the pathogenesis of Parkinson's disease. Functions of NKs are mediated by NK receptors. Substance P, the natural ligand of NK1 receptor, is found to have neuroprotective effects on dopaminergic neurons. Septide is a selective NK1 receptor agonist.

Double immunofluorescence revealed that NK1 receptor immunoreactivity was primarily found in perikarya of striatal interneurons, namely the cholinergic, nitric oxide synthase (NOS)-positive striatal interneurons. On the other hand, double immunofluorescence of the nigral region showed that NK1 receptor immunoreactivity co-localized in dopaminergic, γ -amino-butyrate (GABA) and NOS-immunoreactive neurons.

In 6-hydroxydopamine (6-OHDA)-lesioned rats, an animal model of Parkinson's disease, both striatal and nigral mRNA levels of NK1 receptor of the lesioned side were significantly lower than the non-lesioned side. Immunofluorescence studies showed that NK1 receptor immunoreactivity was up-regulated in striatal interneurons and in nigral GABAergic and NOS-immunoreactive neurons on the lesioned side.

By double immunofluorescence, co-localization of NK1 receptor immunoreactivity in ionotropic glutamate receptors, namely, N-methyl-D-aspartate (NMDA) and α -amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors were observed in the neostriatum and the substantia nigra (SN). This implies roles of NK1 receptor in these excitatory neurons.

In order to investigate the neuroprotective effect of septide *in vitro*, septide (1 μ M) was co-incubated with 6-OHDA (200 μ M) in primary cell cultures of neonatal rat dopaminergic neurons. After 25 hours, massive neuronal cell death was observed in those cultures incubated with 6-OHDA, whereas in septide co-incubation cultures most neurons were seen to be intact. By flow cytometric analysis, 17.03 ± 2.13 % of tyrosine hydroxylase (TH)-immunoreactive neurons were found to survive after co-incubation treatment but only 4.92 ± 1.40 % of TH-positive neurons were found to survive after 6-OHDA treatment. In addition, double immunofluorescence revealed that the level of TH immunoreactivity was also reduced in the surviving neurons after 6-OHDA treatment. No significant reduction of TH immunoreactivity was found in the neurons co-incubated with septide and 6-OHDA. However, significant reduction of NK1 receptor immunoreactivity was found in the neurons co-incubated with septide and 6-OHDA. The present results indicate that septide has neuroprotective effects on dopaminergic neurons in culture. Activation of NK1 receptor by septide

may have implications in treatment of Parkinson's disease.

Further investigations were done on the *in vivo* neuroprotective action of septide in 1-week and 2-week striatal lesioned rats. In apomorphine-induced rotational behavioral test, the numbers of rotations in the striatal lesioned rats after septide treatments were reduced. Besides, TH immunoreactivity in 1-week lesioned neostriatum after septide treatment was partly replenished. Septide restorative effect of TH immunoreactivity was also seen in the SN after 1-week lesion. Concordant with *in vitro* co-incubation of septide and 6-OHDA, NK1 receptor immunoreactivity in the SN of 1-week striatal lesioned rats after septide treatment was diminished as shown by immunofluorescence technique.

Double immunofluorescence results illustrated the changes of distribution patterns of NK1 receptor of different neuronal subtypes and astrocytes in the neostriatum and the SN. In the neostriatum of 1-week striatal lesioned rats, most of the parameters returned back to the level as the normal animal except the NOS immunoreactivity. In the neostriatum of 2-week striatal lesioned rats after septide treatments, most of the parameters remained the same as the lesioned rats, except a reduction of NOS immunoreactivity was observed. In the SN of 1-week striatal lesioned rats after septide treatment, NK1 receptor immunoreactivity in dopaminergic neurons restored back to normal. However, reductions of NK1 receptor immunoreactivity in NOS-immunoreactive neurons and NOS immunoreactivity were observed. In the SN of 2-week striatal lesioned rats after septide treatment, NOS and glial fibrillary acidic protein (GFAP) immunoreactivity was restored back to normal; while, TH immunoreactivity and NK1 receptor immunoreactivity in GABAergic neurons declined. These findings may imply a therapeutic value of septide on the treatment of Parkinson's disease.

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